



APPENDIX VERSION OF AMENDMENTS MARKED TO SHOW CHANGES

RECEIVED
FEB 06 2003
TECH CENTER 160012900

Please replace the paragraph beginning at page 2, line 2 with the following:

--This application is a continuation of Application Serial No. 08/927,368, filed September 11, 1997, now abandoned; which is a continuation-in-part of Application Serial No. 08/899,247, filed July 23, 1997, now abandoned; which is a continuation-in-part of Application Serial No. 08/832,078 filed April 3, 1997, now issued as U.S. Patent No. 6,040,497. --

Please replace the paragraph beginning at page 86, line 11, with the following:

--Glyphosate resistant corn lines GA21, FI117, GG25 and GJ11 were crossed to various inbred lines to facilitate hybrid development as described in example 14. Genomic DNA used for Southern blot analyses was isolated from the resulting backcrossed plants. The backcross populations consisted of plants that were segregating 1:1 for the GA21, FI117, GG25 or GJ11 insertion. Positive and negative GA21 segregants were identified by polymerase chain reaction (PCR) using oligonucleotide primers specific to the pDPG434 fragment used for transformation. Negative segregants served as nontransgenic control plants. The PCR primers used for the analysis spanned the mutant EPSPS-nos junction and generated a 192 bp fragment. The sequence of the upper primer located on the mutant EPSPS gene is 5'-ACGTACGACGACCACAGGATG-3' (SEQ ID NO:1). The sequence of the lower primer located in nos is 5'-GCAAGACCGGCAACAGGATTC-3' (SEQ ID NO:2). Genomic DNA was isolated from positive and negative plants as described in Dellaporta *et al.*, (1983). DNA was isolated from field-grown and greenhouse-grown plants. --

Please replace the paragraph beginning at page 86, line 27 with the following:

--DNA fragments used for probe preparation were isolated by gel-purification of restriction digests of plasmid DNA or were generated by PCR. The mutant EPSPS PCR fragment used as a probe was generated using primers that produce a 324 bp fragment internal to the EPSPS gene. This fragment initiates approximately 400 bp down stream from the start codon. The primer sequences used to generate this fragment are: 5'-TTTGGCTCTTGGGGATGTG-3' (upper) (SEQ ID NO:3) and 5'-TTACGCTAGTCTCGGTCCAT-3' (lower) (SEQ ID NO:4). Probes were labeled with ³²P using the random priming method (Boehringer Mannheim) and purified using Quik-Sep® spin columns (Isolab Inc., Akron, OH). Blots were prehybridized at 65° C for 1-2 hours and hybridized with denatured probe for approximately 18 hours at 65° C. Prehybridization and hybridization solution consisted of 5X SCP, 2X Denhardt's Solution, 0.05 M Tris, pH 8.0, 0.2 % SDS, 10 mM EDTA, 100 mg/l dextran sulfate, and 125 µg/ml denatured salmon sperm DNA. Following hybridization, blots were washed 4 times for 10 min. in 0.25X SCP/0.2% SDS. Membranes were blotted dry and visualized by autoradiography. To reprobe blots, probes were removed by treating blots in 0.05 M NaOH/0.2% SDS for 10 min. followed by neutralization in 0.2 M Tris, pH 7.5/0.2% SDS/0.1 X SCP for 20 minutes at approximately 25° C. --

Please replace the paragraph beginning at page 96, line 24 with the following:

--The presence of a gene in a transformed cell may be detected through the use of polymerase chain reaction (PCR). Using this technique specific fragments of DNA can be

amplified and detected following agarose gel electrophoresis. For example the mutant EPSPS gene may be detected using PCR. Two hundred to 1000 ng genomic DNA is added to a reaction mix containing 10 mM Tris-HCl pH 8.3, 1.5 mM MgCl₂, 50 mM KCl, 0.1 mg/ml gelatin, 200µM each dATP, dCTP, dGTP, dTTP, 0.5µM each forward and reverse DNA primers, 20% glycerol, and 2.5 units Taq DNA polymerase. The primer sequences are (upper) 5'-TTTGGCTCTTGGGGATGTG-3' (SEQ ID NO:3) and (lower) 5'-TTACGCTAGTCTCGGTCCAT-3' (SEQ ID NO:4). The reaction is run in a thermal cycling machine as follows: 3 minutes at 94 C, 39 repeats of the cycle 1 minute at 94 C, 1 minute at 50 C, 30 seconds at 72 C, followed by 5 minutes at 72 C. Twenty µl of each reaction mix is run on a 3.5% NuSieve gel in TBE buffer (90 mM Tris-borate, 2 mM EDTA) at 50V for two to four hours. Using these primers a 324 base pair fragment of the mutant EPSPS transgene is amplified. --



APPENDIX B: PENDING CLAIMS

83. (Amended) A method of plant breeding comprising the steps of:
- (i) planting in pollinating proximity seeds capable of growing into first and second parent maize plants, said first parent maize plant comprising a first EPSPS transgene, wherein said first parent plant is capable of being rendered male-sterile by treatment of said plant with glyphosate, and wherein said first plant is vegetatively and female reproductively tolerant to said treatment with the glyphosate;
 - (ii) cultivating said seeds to produce said first and second parent maize plants;
 - (iii) causing said first parent maize plant to be male-sterile by treating said first parent plant with said glyphosate;
 - (iv) allowing the second maize parent plant to pollinate the first parent maize plant; and
 - (v) harvesting seeds produced on the first parent maize plant.
84. (Amended) The method of claim 83, wherein said second parent maize plant is further defined as comprising a second transformation event, said second plant having vegetative tolerance to said glyphosate.
85. (Amended) The method of claim 84, wherein said second parent plant is still further defined as male reproductively tolerant to said glyphosate.
86. (Amended) The method of claim 85, wherein both said first and said second parent maize plants are treated with said glyphosate.
87. (Amended) The method of claim 86, wherein treating said first and second parent plants comprises an over-the-top application of said glyphosate.
96. (Amended) The method of claim 87, wherein treating comprises an over-the-top application of from 8 ounces per acre to 96 ounces per acre of glyphosate.
97. (Amended) The method of claim 83, wherein said treating is carried out between the V4 and VT stages of development.
98. (Amended) The method of claim 83, wherein the step of causing said first parent plant to be male sterile comprises an application of from 8 ounces per acre to 96 ounces per acre of glyphosate.



APPENDIX B: PENDING CLAIMS

83. (Amended) A method of plant breeding comprising the steps of:
- (i) planting in pollinating proximity seeds capable of growing into first and second parent maize plants, said first parent maize plant comprising a first EPSPS transgene, wherein said first parent plant is capable of being rendered male-sterile by treatment of said plant with glyphosate, and wherein said first plant is vegetatively and female reproductively tolerant to said treatment with the glyphosate;
 - (ii) cultivating said seeds to produce said first and second parent maize plants;
 - (iii) causing said first parent maize plant to be male-sterile by treating said first parent plant with said glyphosate;
 - (iv) allowing the second maize parent plant to pollinate the first parent maize plant; and
 - (v) harvesting seeds produced on the first parent maize plant.
84. (Amended) The method of claim 83, wherein said second parent maize plant is further defined as comprising a second transformation event, said second plant having vegetative tolerance to said glyphosate.
85. (Amended) The method of claim 84, wherein said second parent plant is still further defined as male reproductively tolerant to said glyphosate.
86. (Amended) The method of claim 85, wherein both said first and said second parent maize plants are treated with said glyphosate.
87. (Amended) The method of claim 86, wherein treating said first and second parent plants comprises an over-the-top application of said glyphosate.
96. (Amended) The method of claim 87, wherein treating comprises an over-the-top application of from 8 ounces per acre to 96 ounces per acre of glyphosate.
97. (Amended) The method of claim 83, wherein said treating is carried out between the V4 and VT stages of development.
98. (Amended) The method of claim 83, wherein the step of causing said first parent plant to be male sterile comprises an application of from 8 ounces per acre to 96 ounces per acre of glyphosate.

84. (Amended) The method of claim 83, wherein said second parent maize plant is further defined as comprising a second transformation event[-], said second plant having [~~vegetative~~] vegetative tolerance to said [~~preselected herbicide~~] glyphosate.

85. (Amended) The method of claim 84, wherein said second parent plant is still further defined as male reproductively tolerant to said [~~preselected herbicide~~] glyphosate.

86. (Amended) The method of claim 85, wherein both said first and said second parent maize plants are treated with said [~~preselected herbicide~~] glyphosate.

87. (Amended) The method of claim 86, wherein treating said first and second parent plants comprises an over-the-top application of said [~~preselected herbicide~~] glyphosate.

96. (Amended) The method of claim [94] 87, wherein [~~the step of causing said first parent plant to be male sterile~~] treating comprises an over-the-top application of from 8 ounces per acre to 96 ounces per acre of glyphosate.

97. (Amended) The method of claim [96] 83, wherein said [~~glyphosate is applied~~] treating is carried out between the V4 and VT stages of development.

98. (Amended) The method of claim [95] 83, wherein the step of causing said first parent plant to be male sterile comprises an application of from 8 ounces per acre to 96 ounces per acre of glyphosate.

2.0 REMARKS

Claim 96. The method of claim 98, wherein said glyphosate is applied between the V4 and VT stages of development.

The title of the application has been amended to reflect the subject matter elected for prosecution in the instant case. The specification has been amended to insert information regarding the parent application of the instant case.